

## Supporting information

**Manuscript title:** Passive sampling of SARS-CoV-2 for wastewater surveillance

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**Number of pages:** 8

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**Number of tables:** 2

## 1 Map and population of contributing study sites

This section provides a map (Figure S1) showing the locations and wastewater catchment population for each of the study sites which are in Melbourne and Colac, Victoria, Australia.

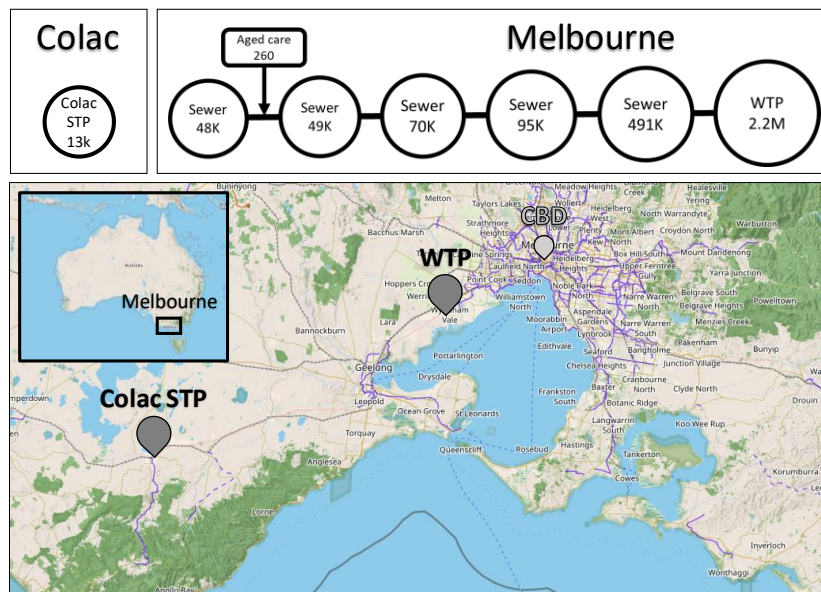


Figure S1. Location of the two study areas: (1) the aged care facility, the trunk sewer sites, and the Western Treatment Plant (WTP) located in Melbourne's metropolitan area within 40km of the Central Business District (CBD), (2) Colac sewage treatment plant (Colac STP), located in regional Victoria 120km from Melbourne's CBD. Map image © OpenStreetMap.

## 2 Extraction and RT-qPCR protocol

**Additional extraction information.** We used a microvolume spectrophotometer (DS-11, DeNovix, USA) to check the approximate concentration and purity of our extracts. Of our samples that had NA concentrations of greater than 50 ng/ $\mu$ L [1] (65%), their  $A_{260}/A_{280}$  ratios were between 2.0 and 2.2 (5<sup>th</sup> and 95<sup>th</sup> percentiles) and hence considered suitable for downstream qPCR [1]. Our samples with concentrations of NA of less than 50 ng/ $\mu$ L (35%) had much more variable  $A_{260}/A_{280}$  ratios ranging from 1.4 to 2.0 (5<sup>th</sup> and 95<sup>th</sup> percentiles), agreeing with [1] that demonstrate that below 50 ng/ $\mu$ L microvolume spectrophotometer readings become more uncertain and variable. Less than 20% of our samples fell below the generally accepted  $A_{260}/A_{280}$  ratios of 1.8 [1], and these samples had estimated NA concentrations of between 4.6 and 39 ng/ $\mu$ L. We still performed qPCR on these samples as it was suspected that the instrument used was not providing accurate  $A_{260}/A_{280}$  ratios at these low NA concentrations [1].

**Additional qPCR information.** The details of the reagents used in our qPCR protocols are provided in Table S1. The parameters used for our thermocycling and qPCR assays are presented in Figure S2. Standard curves for both genes are presented in Figure S3. For further details, please refer to the main body of the report and for specific information about the qPCR kit used, please refer to the manufacturer's guide and [2].

Table S1. RT-qPCR mastermix used in this study.

Reagent name	Volume per reaction
nCoV Reagent A	7.5 $\mu$ L
nCoV Reagent B	1.5 $\mu$ L
nCoV Enzyme Mix	1 $\mu$ L
UltraPure DNase/RNase free water	15 $\mu$ L
RNA template	5 $\mu$ L

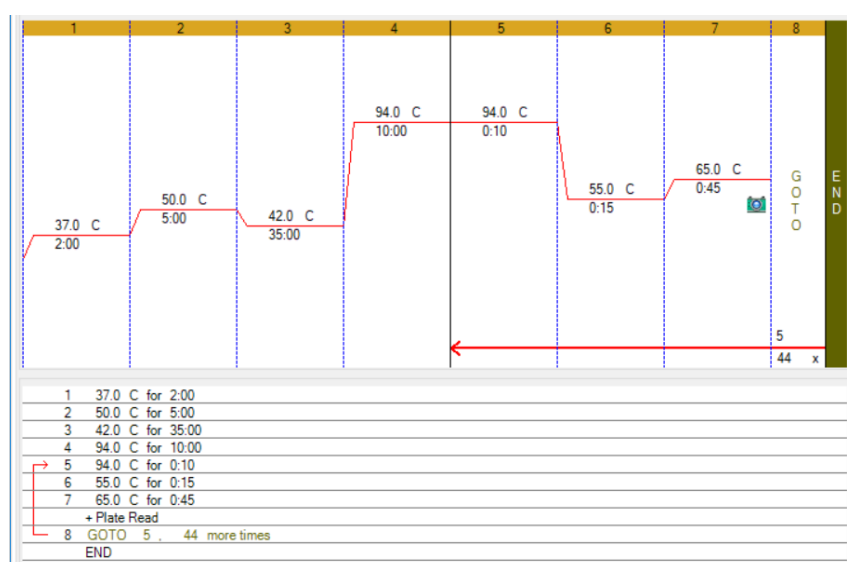


Figure S2. RT qPCR Run for amplification and fluorescence detection on BIORAD CFX 96 (based on Instructions for Perkin Elmer® SARS-CoV-2 Real-time RT-PCR Assay, reaction volume 30  $\mu$ L)

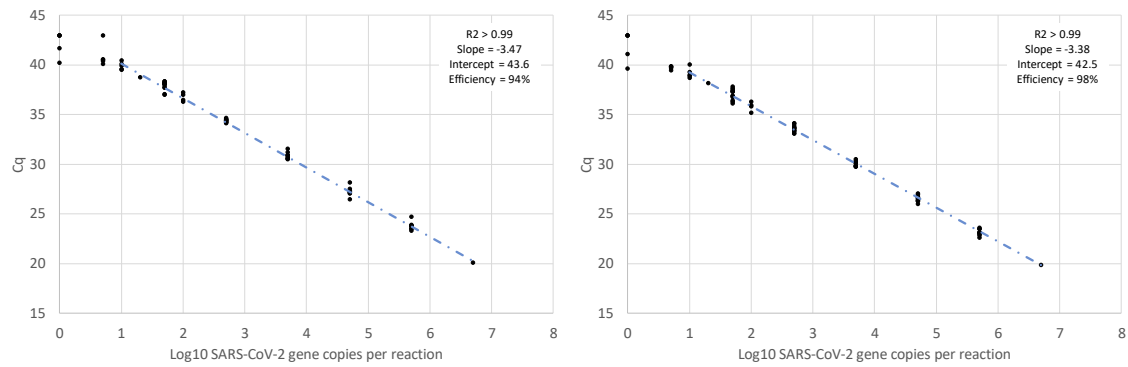


Figure S3. Standard curves for both the *N* gene (FAM – left hand side) and the *ORF1ab* gene (ROX – right hand side). Dots represent individual reactions conducted across the entire experiment ( $n=147$ ), representing a range of spiked concentrations of SARS-CoV-2 from Twist synthetic SARS-CoV-2 RNA control 1 (GenBank ID: MT007544.1, Cat No: 102019). Dashed line represents the most likely estimate of the standard curve for each gene. Also presented are the statistics for the standard curve.

**Limit of quantification (LOQ).** As described in the main body of text, the LOQ was estimated from the replicate standard curves and the supplementary standard dilution as proposed by [3]. We calculated the Coefficient of Variation according to that proposed by [3], following Equation 1.

$$CV = \sqrt{(1 + E)^{(SD)^2 \times \ln(1+E)} - 1} \quad \text{Equation 1}$$

where CV is the coefficient of variation for log-normal distributed data with a qPCR efficiency of  $E$ , and a standard deviation of replicate Cq values.

A plot of CV values versus  $\log_{10}$  reaction concentrations was created (Figure S4), allowing us to visualise at which point the CV decreases below 35% (set to the same threshold as [3]). In this case, the LOQ was observed to be between 5 and 10 copies per reaction and was estimated to be 8.9 copies per reaction using linear interpolation between these datapoints.

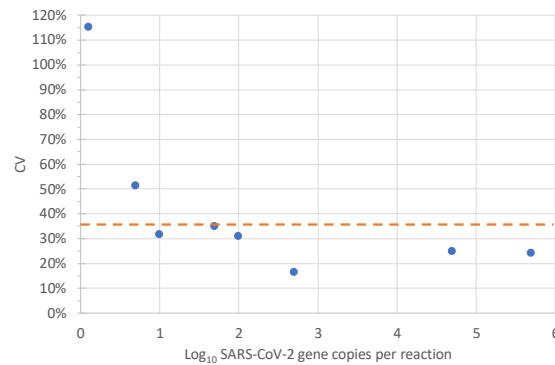


Figure S4. Coefficient of variation for concentrations measured in replicates (via Eq 1) versus the  $\text{Log}_{10}$  gene copies per reaction of SARS-CoV-2. The dashed line represents the 35% threshold set for this study (as per [3]).

### 3 Sequence confirmation methodology

As part of a side-by-side study, we sent 58 qPCR products from passive sampling materials for genomic sequencing. To confirm their identity, each N or Orf1ab amplicon produced was sequenced on Illumina MiSeq (Illumina, USA) using a modified overhang-extension PCR amplicon sequencing protocol. Each amplicon was cleaned before sequencing using the ExoSAP-IT Express PCR Product Clean-up Kit (Applied Biosystems, USA) as per the manufacturers' protocol and extended using an overhang-extension PCR 1. A universal oligonucleotide extension was added to the 5' end of each primer (5' GTGACCTATGAACTCAGGAGTC for CCDC-N-F and CCDC-Orf1ab-F; 5' CTGAGACTTGACATCGCAGC for CCDC-N-R and CCDC-Orf1ab-R). Extension PCR was conducted in a 20 µL reaction containing 1 µL of cleaned amplicon, 10 µL NEB 2x Taq enzyme mix (New England Biolabs, USA), 0.5 µL of the extended forward and reverse primers (at 10 µM master concentration), and 8 µL of nuclease-free water, using the following conditions: 95 °C for 3 minutes, followed by 10 cycles of 95 °C for 15 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds, with a final extension at 72 °C for 7 minutes.

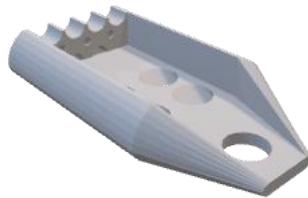
All PCRs were conducted on a Bio-Rad T100 thermal cycler and positive and negative controls were used for all reaction runs and carried through to sequencing. Following amplification, all products were cleaned using NucleoMag (Macherey-Nagel, Germany) NGS beads as per the manufacturer's instructions. Each overhang-extended amplicon was forward and reverse strand index barcoded by sample and sequenced as paired-reads on Illumina MiSeq per the manufacturer's instructions. Upon sequencing, reads were demultiplexed using standard approaches and quality filtered for Phred quality  $\geq 30$ . Filtered reads were mapped to the target amplicon region of the SARS-CoV-2 Wuhan genomic reference sequence (GenBank accession ID: NC045512), requiring a minimum mapping error rate of  $<0.1\%$ , a minimum alignment overlap equivalent to the complete amplicon length (99 and 119 bp for N and Orf1ab respectively), and a minimum sequence identity of 94% using a custom-designed pipeline developed for the Geneious Prime v.2020.2.2 software suite.

## 4 Passive Sampling Unit Designs

This section provides images of the 3D printable passive sampling unit designs. The designs are freely available online for download at [http://www.bosl.com.au/wiki/Passive Sampler](http://www.bosl.com.au/wiki/Passive_Sampler). Three designs are available, the boat-style (Figure S5), the matchbox style (Figure S6) and the torpedo style (Figure S7).



*Figure S5. 3D render of the boat style passive sampling unit.*



*Figure S6. 3D render of the matchbox style passive sampling unit.*



*Figure S7. 3D render of the torpedo style passive sampling unit.*

## 5 Passive sampler loading and wastewater concentrations

Table S2. Loading of SARS-CoV-2 determined for each passive sampler and the average SARS-CoV-2 concentrations in wastewater samples taken using traditional techniques. Note, values are daily averages across all samples taken at each site on each day.

Daily average concentrations using traditional sampling methods [copies/mL]	Average passive sampler loading [copies / passive]
1.25	38.57
13.51	63.16
0.97	63.14
10.37	623.18
0.42	10.00
1.42	40.00
3.55	39.66
0.22	11.69
0.45	1052.87
0.20	10.00
0.61	27.50
0.20	10.00
223.30	49.49
0.33	10.00
2.92	60.86
0.20	11.11
0.20	10.00
0.20	12.61
0.20	10.00
0.44	10.00
1.05	59.84
0.48	17.53
2.06	26.94
1.26	19.32
1.71	32.42
2.44	58.30
0.34	10.00
0.20	10.00
0.21	10.00
0.20	13.44
0.38	11.66
0.58	27.50
0.20	26.83

## References

1. Koetsier, G. and E. Cantor. *A Practical Guide to Analyzing Nucleic Acid Concentration and Purity with Microvolume Spectrophotometers*. 2019 Accessed: May 2021]; Available from: [https://www.neb.com/-/media/nebus/files/application-notes/mvs\\_analysis\\_of\\_na\\_concentration\\_and\\_purity.pdf](https://www.neb.com/-/media/nebus/files/application-notes/mvs_analysis_of_na_concentration_and_purity.pdf).
2. Yan, Y., L. Chang, W. Luo, J. Liu, F. Guo, and L. Wang, *Comparison of Seven Commercial Severe Acute Respiratory Syndrome Coronavirus 2 Nucleic Acid Detection Reagents with Pseudovirus as Quality Control Material*. The Journal of molecular diagnostics : JMD, 2021. **23**(3): p. 300-309.
3. Forootan, A., R. Sjöback, J. Björkman, B. Sjögreen, L. Linz, and M. Kubista, *Methods to determine limit of detection and limit of quantification in quantitative real-time PCR (qPCR)*. Biomolecular detection and quantification, 2017. **12**: p. 1-6.